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Occurrence of Herbicides and Their Degradates in Hawaii's Groundwater

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Hawaii, with its isolated insular location, unrelenting pest pressure, and complex volcanic geology, presents many unique challenges to protecting groundwater from pesticide contamination. The U.S. Environmental Protection Agency (EPA) estimated that about 10% of community water system wells and 4% of rural domestic wells in the U.S. contain at least one pesticide or pesticide degradation product (degradate) at the reporting limits of the survey (EPA 1990). Regular sampling of community water systems show pesticides and pesticide degradates in 68 of 457 drinking water sources in Hawaii (State of Hawaii Department of Health 1996). The previous monitoring primarily focused on fumigants such as ethylene dibromide and trichloropropane, triazine herbicides, and chlorinated insecticides such as dieldrin and lindane. The most frequently reported pesticides in Hawaii's groundwater are fumigants used in pineapple fields, triazine herbicides used in sugarcane fields and termiticides. Agriculture in Hawaii is in a change from plantation to small family farms. Various pesticides have been used to control different pests. A recent study categorized a wide range of pesticides as "leachers" or "non-leachers" for a specific Hawaii hydrogeological setting (Li et al. 1998).

This study was to monitor the occurrence of some herbicides found in groundwater in the continental U.S. (Holden et al. 1992; EPA 1990) in selected wells in Hawaii. These include alachlor, bromacil, dacthal, hexazinone, metolachlor, metribuzin, atrazine, ametryn and simazine and their degradates. Findings in this study have supported the log-transformed attenuation factor index ranking as a practical predication means to assist decision-making (Li et al. 1998).

MATERIALS AND METHODS

Optima grade methanol and ethyl acetate, NaCl, Na_2HPO_4 and NaH_2PO_4 were purchased from Fisher Scientific (Pittsburgh, PA). Immunochemicals, enzyme substrates and buffer capsules were obtained from Sigma (St. Louis, MO). All pesticide standards were purchased from ChemService (West Chester, PA). Distilled water was filtered through a sybron/Barnstedt Nanopure II water system set at 17.8 MΩ-cm.

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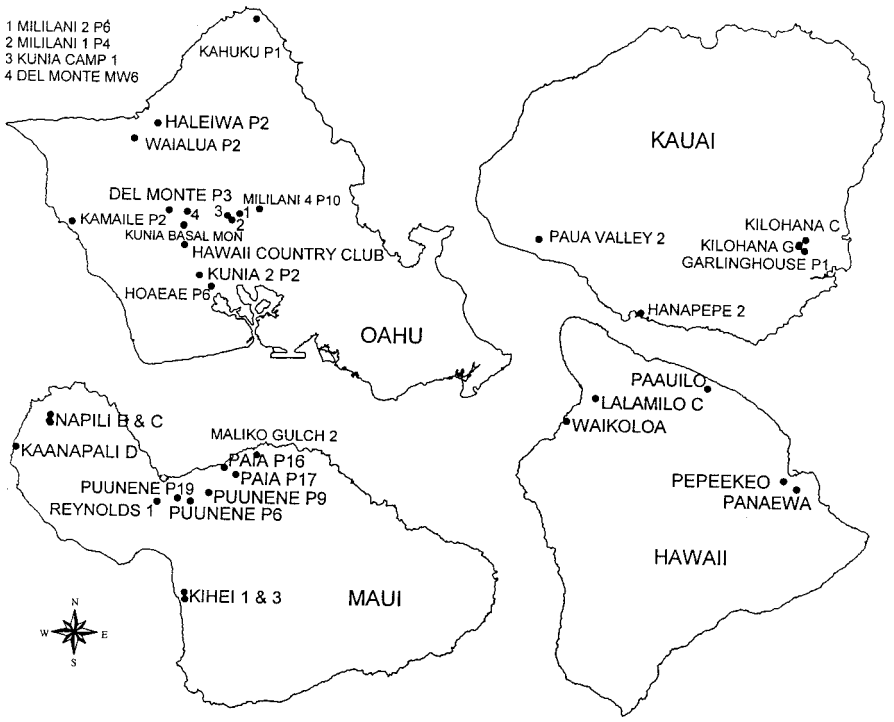

Figure 1. Sampling map. The map is not scaled.

Samples were collected from 5, 5, 12, 14 wells in the Islands of Hawaii, Kauai, Maui and Oahu, respectively, during August 1997 to January 1998 in one-liter amber bottles (Figure 1). There were totally 49 samples including 13 samples collected at a different date within 3 months after the first sampling for confirmation. The wells were selected on a basis of the proximity to areas where herbicides were potentially applied according to the land use. The samples collected from Oahu were delivered to the laboratory in the same day of sampling. The samples from the other islands were in insulated containers with ice packs and were shipped to the laboratory within 24 h after sampling. In both cases, the samples were preserved at approximately 4 °C during transportation. Samples were extracted on the day of arrival or stored at 4 °C for maximum 4 days prior to extraction.

Alachlor, bromacil, hexazinone, metolachlor, metribuzin and the triazines were analyzed according to the EPA method 507 (Munch 1995a), and dacthal according to the EPA method 508 (Munch 1995b). The two methods share the same procedure except using different GC detectors. The extraction method involved adding 100 g of NaCl and 50 mL of phosphate buffer (0.1 M, pH 7) to 1

L of sample. The sample was then extracted with methylene chloride (3 x 60 mL). The extracts were combined and dried over anhydrous Na_2SO_4. The solvent was evaporated with a rotary evaporator set at ambient temperature (23 ± 2 °C) and the coolant circulator at -10 °C, and exchanged to methyl-*t*-butyl ether in a final volume of 5 mL under a gentle stream of nitrogen gas. The final extract was injected into a Hewlett-Packard (HP) 5890 GC equipped with a 30 M DB-5 (J & W Scientific Inc.) or HP-5 fused silica capillary column and the compounds detected with a nitrogen-phosphorus detector and an electron-capture detector. The confirmation column was a 30 M DB-1701 fused silica capillary column. The extracts were held at 4 °C for less than 14 days prior to analysis. The recoveries of the analytes and the surrogates 1,3-dimethyl-nitrobenzene and decachlorobiphenyl were within 93-120%.

Hybridoma culture fluid containing monoclonal mouse anti-atrazine antibodies (AM7B2.1) was kindly provided by Dr. Alexander Karu at the University of California, Berkeley. The atrazine hapten *2h* was a generous gift from Dr. Bruce Hammock at the University of California, Davis. Analyte stock solutions for enzyme linked immunosorbent assays (ELISAs) were prepared in dimethyl sulfoxide (DMSO). Horseradish peroxidase (HRP) tracer was synthesized according to the procedure previously reported (Schneider and Hammock, 1992). ELISAs of each standard and sample in quadruplicate per plate were carried out in 96-well polystyrene plates (MaxiSorp F96, Nalge Nunc Int., Denmark). Affinity-purified goat anti-mouse IgG was diluted in 0.05 M carbonate buffer, pH 9.6 (1:2000) and incubated in the Nunc microplate overnight at 4 °C. The plate was washed 5 times with 0.02 M phosphate-buffered saline (pH 7.5) containing 0.05% Tween 20 (PBST). Subsequently, the hybridoma culture fluid (AM7B2.1) in 0.02 M PBST (1:2000) was added and incubated overnight at 4 °C. The plate was washed 5 times with PBST. One volume of 0.2 M PBST (pH 7.5) was added to nine volume of each water sample to adjust the ionic strength. Atrazine standard solutions in 0.02 M PBST or samples (50 µL) and an equal volume of PBS-diluted HRP tracer (1:20000) were incubated on the plate for 30 min at room temperature. The plate was then washed 5 times with PBST to remove the unbound immunoreactives. An aliquot of 100 µL of substrate solution was added into each well. The substrate solution was 200 µL of tetramethylbenidine (6 mg in 1 mL of DMSO) and 50 µL of 1 % H_2O_2 in 12.5 mL of 0.1 M sodium acetate buffer (pH 5.5). The reaction was stopped after 30 min by adding 50 µL of 4 M H_2SO_4. The absorbance was measured at 450 nm with a Vmax microplate reader (Molecular Devices Corp., Sunnyvale, CA). All inhibition curves were calculated by a four-parameter logistic equation.

RESULTS AND DISCUSSION

The average recoveries of alachlor, atrazine, bromacil, dacthal, hexazinone, metolachlor and metribuzin spiked in groundwater samples ranged from 93 to

120% with standard deviations of 5-16 as determined by GC with DB-5 and DB-1701 columns. Direct analysis of atrazine spiked in groundwater by ELISA also gave a quantitative recovery. The assay showed a half-maximum inhibition (I_{50}) of 25 nM (*i.e.*, 5.4 μg/L) of atrazine. The assay gave moderate cross reactivity (CR) with simazine (28%) and ametryn (15%), low cross reactivity with atrazine degradates (14% or less), and practically no cross reactivity with some commonly used pesticides in Hawaii and their degradates (Table 1). The I_{50}s and cross reactivity patterns were similar with those previously reported using the same antibody and hapten (Karu et al. 1990; Schneider and Hammock 1992). The method detection level (MDL) was 0.1 μg/L of atrazine, defined as 10 times of the background control value.

Table 1. Cross-reactivity of atrazine ELISA

Compound	I_{50} (μM)	CR%
Atrazine	0.0025	100
Deethylatrazine	0.0182	14
Deisopropylatrazine	0.2955	0.83
Diaminoatrazine	16.09	0.02
Hydroxyatrazine	0.0356	6.9
Simazine	0.0087	28
Ametryn	0.0168	15
Bromacil	21.1	<0.01
Metribuzin	>100	<0.01
Hexazinone	35.3	<0.01
Heptachlor epoxide	31.6	<0.01
Lindane	35.3	<0.01
Dieldrin	28.2	<0.01
Diuron	33.1	<0.01
Dacthal	27.5	<0.01
Metolachlor	28.8	<0.01
Alachlor	28.8	<0.01
MBC[1]	28.8	<0.01
Ethylene thiourea	16.4	<0.01

[1] methyl-2-benzimidazolecarbamate.

The ELISA method was used to screen the presence of atrazine in 49 samples from 36 wells. Fifteen samples gave an inhibition equivalent to 0.1 μg/L of atrazine or above (Figure 2). Those 15 positive samples were from Puunene pump 6, puunene pump 16, puunene well 19, Paia well 17, Kihei well 1, Kihei well 3 and Kaanapali well D in Maui, and Pepeekeo and Paauilo wells in Hawaii Island (Figure 1). Thirty-four of the 49 samples, including 9 of the 15 ELISA positive samples, were analyzed by GC to identify the presence of atrazine, deethyl atrazine (DE-atrazine) and simazine. There were no false negatives for atrazine by ELISA. DE-atrazine was detected in 11 of the 34 samples analyzed by GC (Figure 3). GC analyses revealed that the sample (Garlinghouse pump 1) giving an apparent inhibition equivalent to 0.08 μg/L of atrazine by ELISA contained 0.708 μg/L of ametryn (Figure 3). This disagreement between ELISA and GC is apparently due to low cross reactivity (15%) of the assay for ametryn. If the assay cross reactivity is considered, ametryn concentration in the sample would be 0.533 μg/L by ELISA. The results showed that the ELISA was a reliable method for screening atrazine in groundwater samples, but not for other triazine herbicides and their degradates because of the assay specificity.

Forty one samples from the 36 wells were analyzed for possible presence of alachlor, bromacil, dacthal, hexazinone, metolachlor and metribuzin. Bromacil

was found in three wells with concentrations ranging from 0.82 to 2.45 μg/L. The wells contaminated with bromacil were located in Maui (0.82 μg/L), and Oahu (2.24 and 2.45 μg/L) (Figures 1 and 3, Table 2). Hexazinone was found in eight wells with concentrations ranging from 0.13 to 0.99 μg/L. These wells found hexazinone were located in Maui (0.36-0.99 μg/L), Hawaii (0.13 and 0.18 μg/L), and Oahu (0.16 and 0.61 μg/L). Alachlor, dacthal, metolachlor and metribuzin were not found in these samples.

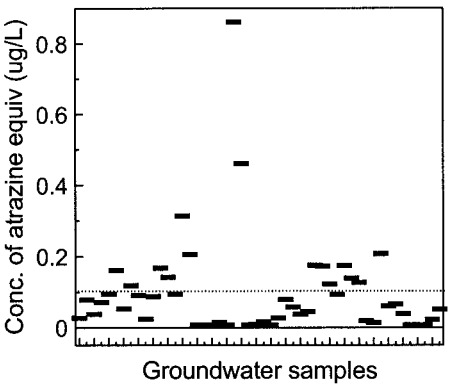

Figure 2. ELISA screening of atrazine in 49 samples from 36 wells. The dotted line is MDL (0.1 ug/L).

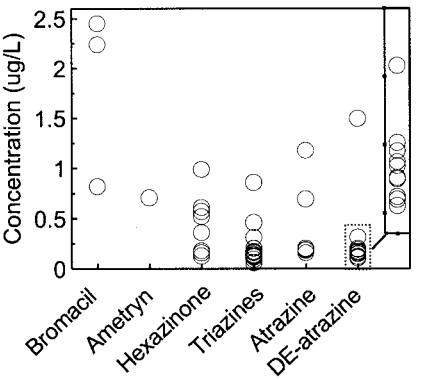

Figure 3. Herbicides and their degradates detected in wells in Hawaii sampled from September 1997 to January 1998.

Table 2. Residues of bromacil and hexazinone found in the samples

Well location	Concentration (μg/L)[1]		Land use
	Bromacil	Hexazinone	
Maui, Paia pump 16	ND	0.36	sugarcane
Maui, Paia pump 17	0.82	0.99	sugarcane, pineapple
Maui, Puunene pump 9	ND	0.52	sugarcane
Maui, Puunene pump 19	ND	0.57	sugarcane
Oahu, Kunia basal monitoring well	2.24	ND	old mixing and storage
Oahu, Kunia camp well 1	2.45	ND	old mixing and storage
Oahu, Del Monte monitoring well 6	ND	0.61	old mixing and storage
Oahu, Waialua pump 2	ND	0.16	old sugarcane
Hawaii, Paauilo	ND	0.18	old sugarcane
Hawaii, Pepeekeo	ND	0.13	old sugarcane

[1] The concentrations were determined by the primary GC column and confirmed by the secondary column. ND = not detected, or lower than MDL.

In this study, the triazine herbicides and their degradates were primarily detected in the wells located in old sugarcane fields as previously reported (State of Hawaii Department of Health 1996). Bromacil and hexazinone were detected in wells in sugarcane fields and in the areas of storage facility (Table 2). The presence of the

herbicides in the wells was obviously related to the source of contamination, which is an well-accepted phenomenon. Presence of bromacil and hexazinone in Hawaii's groundwater showed in this study agreed well with ranking bromacil and hexazinone as the most leachable chemicals of 40 pesticides applied to Rhodic Eutrustoc soil (represented by the Wahiawa soil series) in Hawaii using the attenuation factor (Li et al. 1998). Ametryn was ranked as a probable non-leacher in the same group as diuron which was non-leaching for Hawaii local experience (Li et al. 1998). However, ametryn was detected in one of the 36 wells analyzed by GC, which may be related to the well construction, fractured flow or other connections to surface water sources rather than leaching. Further study and analysis are needed to understand the leaching behavior of ametryn in Hawaii's hydrogeological settings.

Groundwater contamination by pesticides is attributed to the magnitude of the source, opportunities for interception through foliage ground cover or soil, chemical properties and geological conditions. The findings in this study illustrated again the relation of groundwater contamination with the large areas of use, applications directed to soil, improper storage and spills and disposal of pesticides. Pesticide use has evolved considerably over the years. Land use in Hawaii is also changing. Golf courses and small farms are appearing where large sugarcane and pineapple plantations once stood. Managing pesticides to protect groundwater is becoming more challenging. It is essential for pesticide users, manufacturers and regulatory agencies to establish and follow the best management practices to minimize groundwater contamination and to reduce the effects on downstream ecosystems.

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